BULLETIN OF THE
U.S. DEPARTMENT OF AGRICULTURE

No. 92

Contribution from the Bureau of Entomology, L. O. Howard, Chief.
May 15, 1914.

25 MAY. 19
ENTOM.

DESTRUCTION OF GERMS OF INFECTIOUS BEE DISEASES BY HEATING.

By G. F. White, M. D., Ph. D., *Expert, Engaged in the Investigation of Bee Diseases.*

INTRODUCTION.

To reduce the losses due to bee diseases beekeepers have often employed heat in one form or another. The direct flame has been used in scorching or burning the inside of hives that have housed infected colonies. Before being fed back to bees honey is often heated for the purpose of destroying the germs of bee diseases, should any be present. Heat is used in the rendering of wax and in the making of comb foundation. It is natural and very appropriate, therefore, that beekeepers should inquire about the amount of heating that is necessary to destroy the germs that produce diseases among bees.

As no work had been done to determine the facts relative to this question with any degree of accuracy, the writer has performed during the last two years a number of experiments for the purpose of ascertaining them. Of these experiments 55 are summarized in the three tables included in this paper. It may be of interest to beekeepers to know in a general way how these experiments were made. A brief description of the methods used will serve also to make the tables more readily understood. An aqueous suspension of larvæ sick or dead of the disease is made and placed in a small glass tube. This tube is immersed in water of the temperature desired in the heating. After the germ-containing material is heated in this way it must be tested to determine whether or not the germs have been destroyed. In the case of American foul brood this can be done by inoculating a suitable artificial medium with the heated material and observing the presence or absence of growth of *Bacillus larvæ*, the germ of this disease. As there is no artificial medium now known suitable for cultivating the infecting agent of either European foul brood, sacbrood,

Note.—This paper is of interest to beekeepers in all parts of the United States; it was read before the New York State Beekeepers' Association, February 10, 1914, at Ithaca, N. Y.

or Nosema disease, healthy colonies of bees must be inoculated in making the test in case of these diseases. This is done by feeding the bees the heated germ-containing material in sirup. If the disease is produced by this feeding, naturally the infecting agent has not been destroyed by the heating; but if the disease is not produced, it virtually has been destroyed by it. By repeated experiments of this kind in which the temperature used in the heating is varied, the minimum temperature at which any virus is killed can be determined. As will be seen from the tables, 13 experiments for European foul brood, 22 for sacbrood, and 20 for Nosema disease were made in which healthy colonies were inoculated with heated germ-containing material from these three diseases, respectively. In the last disease the stomachs from diseased bees furnished the germ-containing material for heating and feeding. In these experiments the temperature was maintained for 10 minutes as a rule.

DISEASES OF THE BROOD OF BEES.

Nearly a century and a half ago the name "foul brood" was used for a destructive brood disorder of bees, and for almost a century later it was apparently the custom to diagnose as foul brood any destructive disease of the brood. About half a century ago bee-keepers began to note that all of the brood diseases are not the same. They began, therefore, to write of different forms of foul brood. At the present time it is known that there are at least three infectious diseases of the brood of bees. All of these diseases are more or less destructive, and it is quite likely that each of them has now and then been diagnosed as foul brood. In America these brood diseases are now known as European foul brood, American foul brood, and sacbrood.

EUROPEAN FOUL BROOD.

In European foul brood death occurs early, the larvæ dying usually before the time for cell capping. There is no viscidity (ropiness) to the decaying larvæ as a rule, and no pronounced odor present.

Numerous samples of this disease have been examined from the United States, and some from Canada. Its presence also in England, Germany, Switzerland, and Denmark is strongly suggested by written reports from these countries. It is very probable that the disease has a much wider geographical distribution than these facts indicate.

Two years ago the fact was demonstrated that the germ causing European foul brood is the microorganism to which the name *Bacillus pluton* is given. In a paper [1] announcing the fact it was stated that the studies then made indicated that the germ is easily killed by heat. This belief has been confirmed by further experiments.

[1] White, G. F., 1912. The Cause of European Foul Brood. U. S. Dept. Agriculture, Bureau of Entomology, Cir. No. 157.

Table I gives a brief summary of 13 inoculation experiments performed for the purpose of determining approximately the amount of heating necessary to destroy the germ of European foul brood.

TABLE I.—*A summary of the experiments made to determine approximately the minimum amount of heating necessary to destroy the germ causing European foul brood.*

Dates of inoculation.	Temperature.	Time of heating.	Results of inoculation.
	°C.	Min.	
Sept. 12, 1912	75 to 80	10	No disease produced.
Do	65 to 70	10	Do.
Sept. 23, 1912	64 to 66	10	Do.
Oct. 12, 1912	64 to 65	10	Do.
Oct. 1, 1912	62 to 63	10	Do.
Oct. 8, 1912	62 to 63	.....	Do.
Oct. 10, 1912	62 to 63	10	Disease produced.
Oct. 4, 1912	61 to 62	10	Do.
Aug. 8, 1913	60	20	Do.
Sept. 3, 1912	60	10	Do.
Sept. 20, 1912	58 to 60	10	Do.
Sept. 28, 1912	57 to 60	20	Do.
Sept. 20, 1912	55 to 56	10	Do.

It will be observed by an inspection of Table I that European foul brood was produced in every instance where healthy colonies were fed disease material which had been heated for 10 minutes at temperatures below 63° C. (145.4° F.), but that no disease was produced when temperatures higher than 63° C. (145.4° F.) were used for the same length of time. The minimum temperature that can be used, therefore, in destroying the germ of European foul brood, if it is applied for 10 minutes, lies somewhere between 60° C. (140° F.) and 65° C. (149° F.), being near 63° C. (145.4° F.).

AMERICAN FOUL BROOD.

American foul brood is the disease of the brood of bees that is best known to beekeepers and is the one the presence of which they have been able to recognize most easily. In this disease the larvæ usually die after the cells containing them are capped. The disease is characterized especially by the marked viscidity (ropiness) manifested by the decaying larvæ that are dead of the disease. The pronounced odor noticeable within hives housing colonies affected by this disease, especially in its later stages, is another well-known characteristic.

This disease is very widely distributed geographically. Samples of it have been received from many localities in the United States, from Switzerland, New Zealand, Germany, England, and France, and it is very probable that it has a much wider geographical distribution even than is indicated by these facts.

Until seven years ago the cause[1] of American foul brood was not known. At that time the fact was demonstrated positively that the

[1] White, G. F., 1907. The Cause of American Foul Brood. U. S. Dept. of Agriculture, Bureau of Entomology, Cir. No. 94.

germ causing the disease is the one to which the name *Bacillus larvæ* is given.

The facts obtained to date are too meager to justify anything more than a general statement regarding the minimum amount of heating that can be employed in rendering material containing the germ of American foul brood noninfectious. Taking rather wide limits, it may safely be said that the minimum temperature at which this can be done, if the temperature is applied for 10 minutes, lies somewhere between 90° C. (194° F.) and 100° C. (212° F.). It seems quite probable, indeed, that a temperature less than 98° C. (208.4° F.) will suffice if applied for 10 minutes. When 100° C. was used the spores of *Bacillus larvæ* were killed in less than five minutes.

SACBROOD.

Observant beekeepers have for many years noted the presence of dead brood which seemed to them to be different from that dead of foul brood. Some were inclined to believe that the disease was an infectious one; a larger number apparently were disposed to ascribe the trouble to such causes as an unsatisfactory queen, starvation, and the like. This brood disease has been recently demonstrated to be an infectious one, and the name "sacbrood" has been given to it. Larvæ that die of this disease do so almost invariably after the time of cell capping. The most characteristic symptom of the disease is the saclike appearance of the dead larvæ when they are removed from the cell. This fact suggested the name "sacbrood" for the disease.

Sacbrood is frequently met with. Its presence has been diagnosed by Dr. A. H. McCray and the writer in 367 samples received from 44 States of the Union and in 13 samples received from Canada. Reports from England, Switzerland, and Australia indicate strongly that this disease exists in these countries also. It is very probable that it has a much wider geographical distribution than is shown by these facts.

More than a year ago it was again the writer's fortune to determine the cause of another brood disease. Unlike the cause of either European foul brood or American foul brood, the infecting agent causing sacbrood has not yet been seen. It was demonstrated, however, that the infecting agent in this disease passes through the pores of earthenware filters. For this reason the cause of sacbrood is spoken of as a filterable virus.

In a paper[1] announcing the cause of sacbrood the statement is made that the germ causing the disease is destroyed by a comparatively small amount of heat. This belief is confirmed by the results of the experiments summarized in Table II.

[1] White, G. F., 1913. Sacbrood, a Disease of Bees. U. S. Dept. of Agriculture, Bureau of Entomology, Cir. No. 169.

TABLE II.—*A summary of the experiments made to determine approximately the minimum amount of heating necessary to render sacbrood material noninfectious.*

Dates of inoculation.	Temperature.	Time of heating.	Results of inoculation.
	°C.	*Minutes.*	
July 27, 1912	95 to 100	2	No disease produced.
Aug. 8, 1912	95 to 100	2	Do.
Aug. 29, 1912	75 to 80	10	Do.
Sept. 5, 1912	65 to 70	20	Do.
Sept. 3, 1912	55 to 60	20	Do.
Aug. 26, 1913	80	15	Do.
Do.	75	15	Do.
Do.	70	15	Do.
Do.	65	15	Do.
Do.	65	15	Do.
Sept. 2, 1913	65	15	Do.
Sept. 3, 1913	60	20	Do.
Sept. 9, 1913	60	15	Do.
Sept. 10, 1913	60	15	Do.
Sept. 17, 1913	60	10	Do.
Sept. 10, 1913	58	10	Do.
Sept. 17, 1913	58	10	Do.
Sept. 18, 1913	57	10	Sacbrood produced.
Sept. 9, 1913	55	20	Do.
Sept. 10, 1913	55	10	Do.
Sept. 17, 1913	55	10	Do.
Aug 6, 1913	50	30	Do

From Table II it will be observed that when larvæ dead of sacbrood were heated 10 minutes at a temperature of 57° C. (134.6° F.) or less and then fed to a healthy colony, sacbrood was produced; if, on the other hand, the dead larvæ used in making the feeding were heated to 58° C. (136.4° F.) or higher, the disease was not produced. The conclusion to be drawn from these experiments is that the minimum temperature, when maintained for 10 minutes, at which the infecting agent causing sacbrood is destroyed lies somewhere between 55° C. (131° F.) and 60° C. (140° F.), being near 58° C. (136.4° F.).

DISEASES OF ADULT BEES.

Very little is known about the diseases of adult bees. Many names have been used for the purpose of designating them, but the number of such diseases is probably small. There is only one adult disease that can be diagnosed at present by laboratory methods. This one is the Nosema disease.

NOSEMA DISEASE.

Fifty-seven years ago Dr. Dönhoff made a more or less brief study of a disease of adult bees in Germany. He observed that the stomach was the organ that was primarily affected. By feeding to healthy colonies in sirup the crushed stomachs from affected bees Dönhoff demonstrated that the disease could be transmitted to healthy colonies. It was therefore infectious.

The work by Dönhoff had been practically forgotten, apparently, when Zander,[1] of Erlangen, Germany, five years ago observed the

[1] Zander, E., Aug., 1909. Tierische Parasiten als Krankheitserreger bei der Biene. Münchener Bienenzeitung.

presence of a disease among adult bees. From the evidence at hand it seems most probable that the disorder encountered by Dönhoff and the one encountered by Zander are one and the same disease.

Aside from rediscovering the disease Zander has identified the germ causing it as a protozoan (a one-celled animal parasite) and has given to it the name *Nosema apis*. For the disease he has used the name "Nosema Seuche." This is an appropriate one, as it suggests somewhat the nature of the disease. The name "Nosema disease," which the writer suggests as the common name for this disease, is, it will be observed, only a translation of the German name used by Zander.

The germ *Nosema apis* gains entrance to the body of the bee by way of the alimentary canal. In the walls of the stomach the growth and multiplication of the parasite take place to an enormous extent, causing the abnormal appearance manifested by the organ. When the disease reaches an advanced stage the stomach is white and fragile and reveals upon a microscopic examination the presence of the parasite in very large numbers. In the spring of the year, especially, many weak colonies show upon examination a high percentage of Nosema-infected bees. Quite often, indeed, in the examinations that have been made of such colonies, 50 to 90 per cent of the bees in samples taken from them were found to be infected with the parasite. It is an interesting and important fact that a very large number of colonies which are strong and apparently doing well are found upon examination to contain at least a small percentage of Nosema-infected bees.

Nosema apis has a very wide geographic distribution. It has already been encountered in Germany by a number of investigators; it has been found in Australia, Switzerland, and England. The writer has found it in samples of bees received from 27 different States in the United States and in two samples of adult bees from Canada.

From the facts gathered it would seem that many of the cases called "spring dwindling" by the beekeepers are caused, in part at least, by *Nosema apis*. This statement is not by any means to be interpreted as saying that Nosema disease and spring dwindling are always the same.

It has been demonstrated experimentally that colonies can be weakened and killed by feeding to them material containing *Nosema apis*. For this and other reasons it seems certain that the disease causes a loss to apiaries, but, for want of sufficient data, the extent of such loss can not now be estimated at all definitely. From the facts at hand one is justified in at least drawing the conclusion that Nosema infection in a colony tends to weaken the colony. *Nosema apis* is therefore a germ in which the beekeeper is economically interested.

For the purpose of determining approximately the minimum amount of heating that is sufficient to destroy the germ *Nosema apis* the inoculation experiments summarized in Table III were made.

TABLE III.—*Summary of experiments in which the germ, Nosema apis, was heated and fed to healthy colonies.*

Dates of inoculation.	Temperature used in heating.	Time of heating.	Results of inoculation.
	°C.	*Minutes.*	
Oct. 29, 1912	95 to 100	5	No Nosema infection produced.
Nov. 12, 1912	95 to 100	5	Do.
Oct. 29, 1912	80	20	Do.
Nov. 9, 1912	80	10	Do.
Nov. 11, 1912	68 to 70	10	Do.
Do	68 to 70	10	Do.
Nov. 12, 1912	65	20	Do.
Jan. 8, 1913	65	10	Do.
Nov. 11, 1912	60	10	Do.
Do	60	10	Do.
Nov. 20, 1912	60	10	Do.
Feb. 8, 1913	58	10	Do.
Oct. 4, 1913	58	10	Do.
Feb. 8, 1913	57 to 58	15	Do.
Oct. 15, 1913	57	10	Do.
Do	57	10	Do.
Oct. 4, 1913	56	10	Nosema infection produced.
Oct. 15, 1913	56	10	Do.
Jan. 8, 1913	55	20	Do.
Jan. 31, 1913	55	10	Do.

It will be observed from Table III that when *Nosema apis* was heated to 57° C. (134.6° F.) or higher for 10 minutes and fed to healthy bees no infection took place, but when held at temperatures below 57° C. (134.6° F.) for the same period of time the bees became Nosema infected. It is shown, therefore, that the minimum temperature that will destroy the germ *Nosema apis* in 10 minutes lies somewhere between 55° C. (131° F.) and 60° C. (140° F.), being quite near 57° C. (134.6° F.).

By way of parenthesis it might be well to say a word or two further regarding Nosema disease. The studies of this disease disclose the interesting fact that it is not a new one in American apiaries. There is no cause, therefore, for anticipating any additional losses to our apiaries. Indeed, since the presence of the disease is known, hopes may be entertained that methods will be determined for reducing the losses due to it. Considerable work must yet be done, however, before methods for its control can be recommended.

Nosema disease is being studied in England, Germany, Switzerland, and Australia. During the last two years the writer has devoted considerable time to its study in America. The plan is to continue the studies during the present year, after which it is hoped a further discussion of this disease will be justified.

SUMMARY AND GENERAL REMARKS.

The results of these experiments show that when they are maintained for 10 minutes the minimum temperatures that can be used for destroying the germs of the four bee diseases now known to be infectious are as follows:

(1) The minimum temperature for European foul brood lies somewhere between 60° C. (140° F.) and 65° C. (149° F.), being approximately 63° C. (145.4° F.).

(2) The minimum temperature for American foul brood lies somewhere between 90° C. (194° F.) and 100° C. (212°) F., being probably less than 98° C. (208.4° F.).

(3) The minimum temperature for sacbrood lies somewhere between 55° C. (131° F.) and 60° C. (140° F.), being approximately 58° C. (136.4° F.).

(4) The minimum temperature for Nosema disease lies between 55° C. (131° F.) and 60° C. (140° F.), being approximately 57° C. (134.6° F.).

It will be noted, therefore, that 63° C. (145.4° F.) for European foul brood, 98° C. (208.4° F.) for American foul brood, 58° C. (136.4° F.) for sacbrood, and 57° C. (134.6° F.) for Nosema disease are the approximate minimum temperatures at which the germs of these diseases, respectively, are destroyed. Since there are varying factors in experiments of this nature that tend to produce slight variations in results, these temperatures are referred to as being approximate. It is probable that future experiments may cause slight changes to be made in these conclusions. Nothing more than a comparatively slight variation is to be expected, however. In practice the beekeeper, in destroying these germs by heating, will naturally use a quantity of heat somewhat in excess of the minimum amount that is absolutely necessary.

Some generalizations may now be made which will be of interest to the beekeeper. The melting point of beeswax is between 62° C. (143.6° F.) and 64° C. (147.2° F.), inclusive. It will be observed that this same temperature in 10 minutes will destroy the germ causing European foul brood, and that it is about 10° F. above that which will destroy the germs of sacbrood and Nosema disease. A further interesting generalization may be made concerning the heating of honey. Honey when heated to 160° F. reaches a temperature 15° F. above the temperature necessary to destroy the germ of European foul brood and about 25° F. above the temperature that will destroy the infecting agents of sacbrood and Nosema disease. The infecting agents of these three diseases of the bee, therefore, will be destroyed when the temperature of 160° F. is used in the commercial handling of honey. Finally, it is believed that the results of this work on the thermal death point of the viruses of the bee diseases will be directly applicable to the control of these diseases.

○